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Neoalbatrellus subcaeruleoporus sp. nov. (*Scutigeraceae*) from western North America

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Abstract –*Neoalbatrellus subcaeruleoporus* is described as a new species. Its systematic position is supported by molecular and morphological analyses, and a key to the three *Neoalbatrellus* species is provided. A revised key to American and European species of *Scutiger* sensu lato (*Albatrellopsis*, *Albatrellus*, *Laeticutis*, *Neoalbatrellus*, *Polypus*, *Polyporoletus*, *Polyporopsis*, *Scutiger* s.s., *Xanthoporus*, and *Xeroceps*) is appended.

Keywords –*Albatrellaceae*, *caeruleoporus,* correct family, genetic, *Russulales*

Introduction

The genus *Neoalbatrellus* was proposed by Audet (2010) and is distinguished from *Albatrellus* by the hymeniderm and the blue, black and brown colors of the basidiomata. Two species have been placed in *Neoalbatrellus*: *N. caeruleoporus* and *N. yasudae* (Audet 2010). Their trophic status is poorly understood and specific ectomycorrhizal host trees have not been confirmed, although *N. caeruleoporus* is often found under *Tsuga* (Gilbertson & Ryvarden 1986). The presence of extracellular laccase has been reported in basidiomata of *N. caeruleoporus* (Marr et al. 1986). The closest genus to *Neoalbatrellus* is *Albatrellopsis,* based on molecular (ITS) and the dimeric meroterpenoid pigment, grifolinone B (Zhou & Liu 2010). The correct family for these genera is *Scutigeraceae* Bondartsev & Singer ex Singer 1969, a name that has priority over *Albatrellaceae* Nuss 1980 (Audet 2010: 439).

In this paper a new species, *Neoalbatrellus subcaeruleoporus*, is described from western North America. In the past it has been confused with *N. caeruleoporus* due to similarity in basidioma color, but the two species differ both morphologically and genetically.

Table 1: Species names, vouchers, and GenBank accession numbers of the sequences used in the phylogenetic analysis.

Revised name	Deposited name	Voucher	ITS sequence
Albatrellopsis flettii	*Albatrellus flettii*	MICH AHS82164	AY621802
Neoalbatrellus caeruleoporus	*Albatrellus caeruleoporus*	MICH K.A. Harrison 8825	AY963565
Neoalbatrellus subcaeruleoporus	*Albatrellus caeruleoporus*	OSC 66097	KC985126
Neoalbatrellus subcaeruleoporus	*Albatrellus caeruleoporus*	DAVFP 28304	KC862265
Neoalbatrellus yasudae	*Albatrellus yasudae*	HKAS 357766	(Zheng 2006)

Materials & methods

This study is based on collections of *Neoalbatrellus* from western and eastern North America. The specimens were studied macroscopically, microscopically, and genetically. Color terms for basidiomata beginning with a capital letter (e.g. Cadet Gray) follow Ridgway (1912). Unless otherwise specified, the macroscopic description is based on Luther's notes from fresh material

Microscopic features were described from dried specimens (with herbarium acronyms following Thiers 2013). Basidiospores and basidia were observed using bright field microscopy at a magnification of 1250×. Melzer's reagent or IKI was used for microscopic observations of basidiospores. Basidiospores were measured in profile view with the extreme values indicated in parentheses. The number of measurements is cited as n = x/y, indicating x measurements of spores from y specimens. 100 spores were measured for all collections of *N. subcaeruleoporus* studied. The following abbreviations are used: Q = the quotient of spore length and spore width in any one spore, indicated as a range of variation in n spores measured; L_m = mean spore length (µm), W_m = mean spore width (µm), Q_m = quotient of mean spore length and mean spore width (L_m/W_m ratio).

Hyphae, basidia, and spores were observed in cotton blue in lactic acid to verify the presence of gloeoplerous hyphae and cyanophilous (CB+) elements. The cyanophilous thin-walled hyphae appear blue in mass.

The protocols for DNA extraction and amplification of the ITS1+5.8S+ITS2 region in *N. subcaeruleoporus* were done by R.S. Winder and M. Gordon, who provided two *subcaeruleoporus* sequences. Winder slightly modified the method of Kranabetter et al. (2013) by not soaking the sample overnight but by freezing a 50 mg portion of the sample in liquid N and grinding in a sterile mortar and pestle before adding the CTAB buffer. For PCR amplification, the universal primer pair ITS5/ITS4 was used (White et al. 1990; Gardes & Bruns 1993); the PCR product was sequenced by the Genome Sequencing and Genotyping Platform (CHUL Medical Research Centre (Quebec, Canada) using capillary electrophoresis on an ABI 3730/XL Analyzer (Applied Biosystems). Gordon extracted DNA from 50 mg of dried mushroom using the UltraClean DNA Isolation

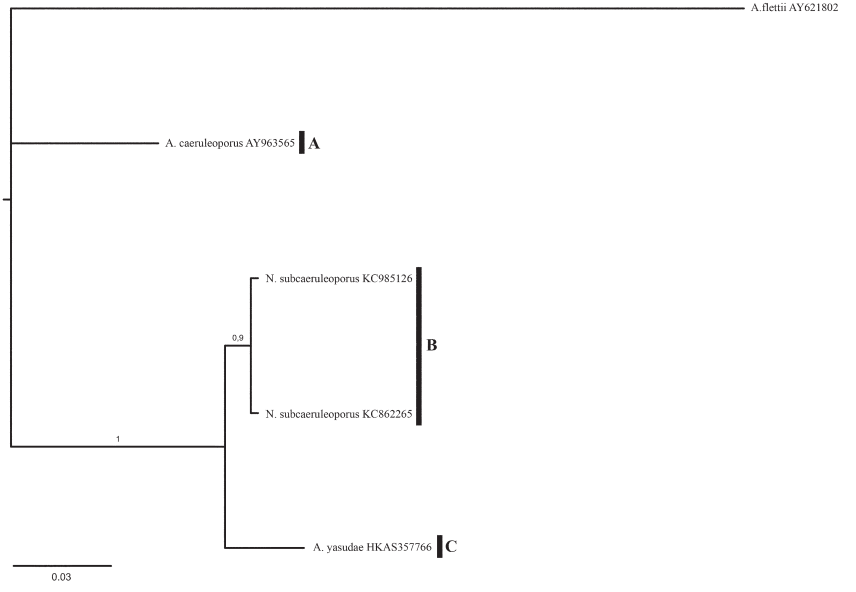

Figure 1: rDNA ITS1+5.8S+ITS2 strict consensus tree as generated using MrBayes (Huelsenbeck & Ronquist 2001; 1,000,000 generations). A: *Neoalbatrellus caeruleoporus*; B: *Neoalbatrellus subcaeruleoporus*; C: *Neoalbatrellus yasudae;* outgroup: *Albatrellopsis flettii.*

Kit (MoBio Laboratories, Inc.) and amplified the ITS region following a standard PCR protocol using primers ITS1f and ITS4 with an anneal temperature of 58°C. The ITS amplicon was sequenced at Eurofins MWG Operon (operon.com).

The sequences used in the phylogenetic analysis are presented in Table 1. *Albatrellus flettii* [≡ *Albatrellopsis flettii*] served as outgroup.

Taxonomy

The phylogeny inferred from the ribosomal ITS1-5.8S-ITS2 sequences from three samples labeled *Albatrellus caeruleoporus* and one each of *A. flettii* and *A. yasudae* is shown in Fig. 1. One *A. caeruleoporus* sequence (AY963565) from eastern North America formed a separate clade (A). Two *caeruleoporus* sequences (KC985126, KC862265) from British Columbia and California formed a distinct and strongly supported clade (B). The sequence labeled *A. yasudae* (HKAS357766) from China formed a third clade (C) separated from the two *A. caeruleoporus* clades. The samples in clade B are considered as representing an independent taxon, described here as *Neoalbatrellus subcaeruleoporus*. We obtained nearly a complete ribosomal ITS sequence from our *N. subcaeruleoporus* holotype and alignment supports it as a new species.

Neoalbatrellus subcaeruleoporus Audet & B.S. Luther, **sp. nov.** Figs 2a, 3

MycoBank MB801480

Differs from *Neoalbatrellus caeruleoporus* by its paler blue fresh basidiomata, its gloeoplerous hyphae, its smaller basidia and basidiospores, and its western North American distribution.

Type: USA. Washington state, Yakima Co., Bumping Lake Road, in William O. Douglas Wilderness across the footbridge from Soda Springs Campground, a.s.l. 918 m, in conifer duff and forest litter directly under *Alnus* sp., with *Abies grandis* and *Pseudotsuga menziesii* nearby, 7 October 1995, coll. B.S. Luther 1995-107-1, det. S. Audet (**Holotype**, WTU-F-50696).

Etymology: from the bluish colors shared with *Neoalbatrellus caeruleoporus*.

Pileus 3–6.5 cm wide × 3–5.5 mm thick, umbonate to convex, irregularly plane or more frequently depressed, orbicular, flabelliform, dimidiate or reniform, firm when fresh, drying hard and brown, black laccate, or greenish–brown and sometimes resinous reddish in areas; surface irregular, dry, glabrous to finely rugose or subtomentose; color a Pale Glaucous Blue at first, later Cadet Gray to Light Mineral Gray with margin often paling to Pale Smoke Gray to Light Olive Gray, when drying reddish undertones developing with ochraceous–orange or orangish areas showing in pileal cracks or where damaged (rodent bites, etc.); margin uniform, convoluted or irregular and inrolled, with lobes or undulations and splits (clefts), concolorous to generally much paler than the pileus, with orangish or ochraceous tints in age, surface glabrous and soft or breaking up into minute scales that are darker than the underlying tissue and slightly roughened, with an appendiculate, sterile edge, when mature prominently incurved or slightly involute and somewhat even; context ≤1 cm thick, white at first, slowly Pale Ochraceous Buff along margins, then

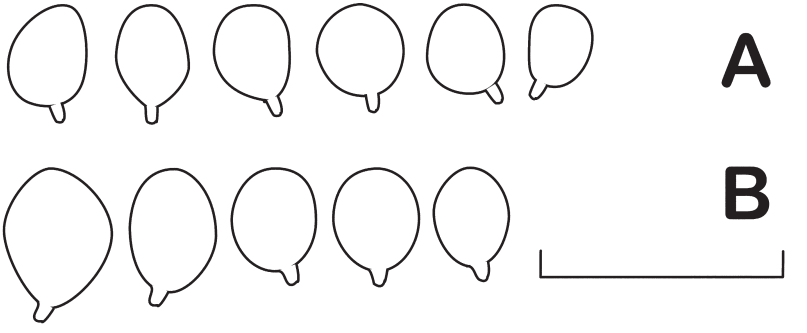

Figure 2. Basidiospores (Scale bar = 9.2 µm).
A. *Neoalbatrellus subcaeruleoporus* (holotype). B. *Neoalbatrellus caeruleoporus* (isotype).

Figure 3. *Neoalbatrellus subcaeruleoporus* (WTU-F-50695).
Fresh basiodiomes (one cut to show context).

Light Pinkish Cinnamon or Light Vinaceous Cinnamon; when dried cinnabar necropigments light, becoming darker after extended storage; odor sweetly fungoid or not distinctive; taste mild and slightly sweetish to slightly peppery. Pores 1–3 per mm (3–6 per mm in dried OSC 66097), orbicular, elongate to slightly angular, pruinose inside, with orange–pinkish necropigments; near the stipe uniform, ≤1 mm wide, concolorous with or paler than the pileus, smaller at the growing margin, uniformly pale bluish and with pale ochraceous or pale orangish to rusty areas at maturity or when injured/bruised, very pale when dry. Tubes decurrent, shallow, 1–2 mm deep, dissepiment walls paler (very pale when dry), fimbriate or granulose. Stipe 3–6 × 1–2 cm, central to strongly eccentric, tapering noticeably toward the base and widening/enlarging toward the pileus, somewhat irregular to slightly twisted, curved or bent, surface smooth to innately fibrillose streaked or with very fine scaly patches, generally sub-cylindric or slightly flattened, fasciculate; surface color concolorous with the pileus, paler and often with very slight pale pinkish overcast tones, or becoming slightly darker or grayer toward the extreme base or below, but paler and almost whitish near the base where starting to taper and beneath where forest duff has adhered to the base, in age with orangish or ochraceous tints; when dried resinous reddish-brown in some areas; context solid in cross-section, pallid when freshly cut, turning pale pinkish very slowly.

Hyphal system monomitic. Pileipellis hymeniderm, hyphae in a tight palisade covered with a brownish resin, narrowly clavate with ends sometimes inflated to 7.1 µm diam., refractive, hyaline to yellowish in IKI, inamyloid with weakly cyanophilous walls. Stipitipellis hyphae similar. Contextual hyphae 2.8–11.3 µm diam. (larger hyphae often ramified and with variable diameters), simple-septate, hyaline, thin-walled, inamyloid, walls weakly cyanophilous; gloeoplerous hyphae occasional, ramified and flexuous, refractive and pale yellow in IKI, intense blue in cotton blue (CB+, cyanophilous). Tramal hyphae 2.8–3.5 µm diam., simple-septate, hyaline, thin-walled, inamyloid, walls weakly cyanophilous, often flexuous and ramified, sometimes with perpendicular branching before or near septa; gloeoplerous hyphae refractive and yellow in IKI, intense blue (cyanophilous) in cotton blue. Basidia 25–33 × 6–7 µm, narrowly clavate, wall weakly cyanophilous, contents blue with yellowish brown inclusions in cotton blue; sterigmata four, straight or slightly curved, ≤4.8 µm long. Basidiospores amygdaliform, ellipsoid to subglobose, hyaline, thin-walled, smooth, inamyloid, weakly to moderately cyanophilous, sometimes with a large brownish-yellow guttule in cotton blue, with a relatively long apiculus, (3.3–)3.6–4.7(–5.1) × (2.8–)2.9–3.7(–3.9) µm, n = 100/4; L_m × W_m = 4 × 3.2 µm; Q = 1.1–1.4(–1.5); Q_m = 1.3; half of the spores with a flat to slightly convex adaxial side.

Macrochemical reactions on context of fresh pileus stipe context: KOH (3%): Pale Pinkish Cinnamon to Light Pinkish Cinnamon, darkening the longer it sits in the reagent. NH_4OH (30%): very pale or similar to KOH reactivity. Ferric sulfate: a distinctive reddish-brown exudate that completely darkens the solution around the fresh sample. Tincture of guaiac resin: –. Phenol: –. Aniline: –. PDAB(4-dimethyl amino benzaldehyde): + (striking bright turquoise after 5 minutes).

Additional specimens examined (all as ***Albatrellus caeruleoporus***): **U.S.A. Washington State**: **King Co.**, Lake Quinault, Rain Forest Mushroom Festival, 18 October 2008, Steve Trudell (WTU-F-13070); **Pierce Co.**, Mt. Rainier National Park, Carbon River area, 15 October 1987, B.S. Luther 1987-1015-1 (WTU-F-50684); **Skamania Co.**, Gifford Pinchot National Forest, Curly Creek Falls Trailhead, 5 October 2008, W. Foster SKAWA001 (WTU-F-13071); **Yakima Co.**, Bumping Lake Road, William O. Douglas Wilderness from Soda Springs Campground, 1 October 1994, B.S. Luther 1994-101-1 (WTU-F-50695); 23 September 1995, B.S. Luther 1995-923-1 (WTU-F-50681). **Oregon**: **Lane Co.**, JM Honeyman Park, 12 November 1992, George Barron (DAOM 215968); **California**: **Humboldt Co.**, Redwood National Park ("Prairie Creek"), UTM N: 415268, UTM E: 4582445 zone 10, 12 December 1997, T. O'Dell 4550 & M. Madsen (OSC 66097). **CANADA. British Columbia**: (**Mainland**) N of Clearwater, near Spahats, 10 September 1982, T. Goward (DAOM 194800); Mt. Elphinstone, Roberts Creek, 29 September 2000, Paul Kroeger (DAOM 229493); **Vancouver Island,** just west of Sooke, close to Juan de Fuca Strait, second growth, 28

October 2010, P. Hutchinson & K. Trim (DAVFP 28293); Sooke, Otter Point Road, 22 November 2011, K. Trim (DAVFP 28304).

Ecology & Distribution— Arising from deep in forest duff during mid-September to mid-December. Associated with *Abies grandis*, *Pseudotsuga menziesii*, *Alnus* sp., *Picea sitchensis, Sequoia sempervirens, Tsuga heterophylla*. It fruited in an arc around the base of *Alnus* sp. but conifers were also close by and mixed in. Rare in western North America in northwestern California, central and southwestern British Columbia, western Oregon, and northwestern and central Washington on the east slopes of the Cascades.

Other specimens examined: ***Neoalbatrellus caeruleoporus:*** **CANADA. Québec**: Quebec, 1999, det. R. Labbé (QFB-8563); Richmond County, Kingsbury, 1984, det. R. Cauchon (QFB-16558L); Beauce, Sainte-Clothilde, late August 2011, coll. M. Nadeau, det. R. Labbé (WTU-F-50683); **Nova Scotia**: Annapolis Co., Bishop Mt. Road, 19 October 1969, coll. & det. K.A. Harrison (MICH 00073662; KAH 8825). **U.S.A. New York State**: near Oneonta, 1963, det. S. Smith (QFB-199L,); Duchess County, Copake, October, leg. & det. C.H. Peck (NY 00730549, isotype of *Polyporus caeruleoporus*).

Remarks: *Neoalbatrellus subcaeruleoporus* is considered rare and has been listed (as *Albatrellus caeruleoporus*) for protection and received special conservation status in the United States (Molina 2008). Also as *A. caeruleoporus,* it has been cited as a possible indicator of western old growth forests and considered in forest management plans (Ginns 1997).

A detailed comparison of *Neoalbatrellus subcaeruleoporus* and *N. caeruleoporus* is presented in Table 2.

The pores of *N. subcaeruleoporus* are somewhat smaller and orbicular to angular with a thicker wall than those of *N. caeruleoporus*. Our *N. subcaeruleoporus* basidiospore measurements are slightly smaller than those given by Ginns (1997, as *A. caeruleoporus*). The dull colors (bluish-gray, with a tinge of orange) not seen in the very young, actively growing basidiomata and appearing only in fully mature specimens, are typical of basidiomata collected late in the season.

It is interesting to note that *N. yasudae* is characterized by basidiospore sizes intermediate (4–5.5 × 3.5–5 µm; Zheng & Liu 2008) between *N. subcaeruleoporus* (3.6–4.7 × 2.9–3.7 µm; Table 2) and *N. caeruleoporus* (4.6–5.6 × 3.8–4.3 µm; Table 2) but shares with *N. subcaeruleoporus* a pileipellis covered with a brownish resinous substance (as seen under the microscope) and a pale hymenophore. These morphological distinctions are supported by ITS sequence analysis.

Concerning macrochemical reactions, ferric sulfate in solution is not as reactive as ferrous sulfate. The strong reaction of the *N. subcaeruleoporus* context in ferric sulfate suggests that ferrous sulfate will likely have a stronger reaction

Table 2: Comparison between *Neoalbatrellus subcaeruleoporus* and *N. caeruleoporus*

Character	*N. subcaeruleoporus*	*N. caeruleoporus*
Cap, dry	Becoming brown or brown to black, with tubes and context over time turning very slowly cinnabar orange	Becoming brownish, with the entire basidiome eventually often turning cinnabar orange
Cap, fresh	≤6 cm in diameter	≤15 cm in diameter [1]
	Light blue to pale blue, often glaucous with reddish orange-reas where damaged, with age, or in cracks	Grayish blue to violaceous, in age tan to light ochraceous brown [2]
Context, in herbarium	White to pale orange (when exposed)	Typically pale orange (also pallid or orange pink) [2]
Context, fresh	White to pale wood brown; slowly discoloring after a few hours in open air to pale ochraceous buff, light pinkish cinnamon, or light vinaceous cinnamon	White [2]
Tubes, fresh	0.7–2 mm long	2–5 mm long [1]
	Concolorous with the cap or paler, becoming orangish or rusty irregularly or where damaged	Grayish blue [2]
Stipe, fresh	Without lateral stipe	With lateral stipe when young
	Concolorous with the pileus or paler; context bruising orange-brown	Deep indigo-blue [1]
KOH on context	Pale or light pinkish cinnamon, darkening over time (3%)	Not discoloring (2%) [2]
30% NH_4OH on context	Very pale or as above (KOH)	Not tested
Phenol on context	Negative	Not tested
Analine on context	Negative	Not tested
Ferric sulfate ($Fe_2(SO_4)_3$)	Context reddish-brown color	Not tested
Ferrous sulfate ($FeSO_4$)	Not tested	Context staining pale yellow [2]
Tincture of guaiac resin (on context)	Negative	Slowly positive blue reaction (≥30 minutes) when tincture replenished
Syringaldazine (laccase test)	Not tested	Reddish (positive: 0.5/4) [4]
Gloeoplerous hyphae	Scattered in context (trama) [3]	Not seen [2]
Basidia	23–32(–44) × 5.6–7.4 µm [3]	33–48 × 6–8 µm [2]
Basidiospore apiculus	Relatively long, tapered [3]	Small, but obvious [2]
Basidiospores	4.0–4.8(–5) × 3.4–3.6(–4) µm [3]	4.8–5.6 × 3.8–4.2(–4.8) µm [2]
	3.6–4.7 × 2.9–3.7 µm, n = 100/4 (DAVFP 28293; OSC 66097; WTU-F-50696; WTU-F-50684)	4.6–5.6 × 3.8–4.3 µm, n = 40/2 (NY 00730549; MICH 00073662)

[1] Overholts (1953); [2] Ginns (1994); [3] Ginns (1997, as *Albatrellus caeruleoporus*); [4] Marr et al. (1986).

on *N. subcaeruleoporus* than on *N. caeruleoporus*. It is interesting to note that *N. caeruleoporus* gives a slow positive reaction with an alcoholic solution of guaiac resin, but *N. subcaeruleoporus* does not. The positive reaction with this reagent simply shows the presence of extracellular phenol-oxidases (tyrosinase, laccase, and peroxidase) and is not specific for a particular enzyme (Gilbertson et al. 1975). This reaction is consistent with a study by Marr et al. (1986) who show a positive laccase reaction with syringaldazine for *N. caeruleoporus.*

Key to species of *Neoalbatrellus*

1. Pileipellis with densely agglutinated pyriform cells ..... ***N. yasudae***
   Pileipellis without densely agglutinated pyriform cells **2**
2. Basidioma pale blue; western North American distribution;
   spores 3.6–4.7 × 2.9–3.7 µm ..... ***N. subcaeruleoporus***
   Basidioma blue; eastern North American distribution;
   spores 4.6–5.6 × 3.8–4.3 µm ..... ***N. caeruleoporus***

Summary

The genus *Neoalbatrellus* has an additional species, *N. subcaeruleoporus*. It is separated from *N. caeruleoporus* by its pale blue basidioma, shorter basidia and basidiospores, lack of a phenol-oxidase reaction, and a western North American distribution, as well as by its DNA sequences.

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Appendix 1. Key to American and European species of *Scutiger* s. lat.
(From Audet 2010; revised by Serge Audet & Georges Fannechere)

1. Clamp connections on contextual generative hyphae numerous 2
   Clamp connections on contextual generative hyphae absent or very rare 15
2. Spores ≤12(−14) µm long 3
   Spores smaller or equal to 7 µm long 10
3. Spores without double wall 4
   Spores with double wall 7
4. Spores not primarily tear-drop shaped 5
   Spores primarily tear-drop shaped 6
5. Pileus brown (fresh) or dark violet (dried) ..... ***Xeroceps skamania***
   Pileus yellow (fresh) or pinkish white to pale red (dried) .... ***Xeroceps yunnanensis***
6. Pileus greenish olive to milk chocolate brown ..... ***Scutiger ellisii***
   Pileus dark brown ..... ***Scutiger pes-caprae***
7. Hyphal system dimitic ..... ***Polyporopsis mexicana***
   Hyphal system monomitic 8
8. Spores c. 9.6–12.6 µm diam. ..... ***Polyporoletus sublividus* s. lat.**
   Spores c. 7.2–10.8 µm diam. 9
9. Basidiomes solitary and with bulbous stipe ..... ***Polyporoletus bulbosus***
   Basidiomes caespitose and with no bulbous stipe ..... ***Polyporoletus sylvestris***
10. Hymenophore entirely yellow when young 11
    Hymenophore not entirely yellow when young 12
11. Some thick-walled hyphae (often >0.5 µm) near the surface from stipe base; spores small (3.5–4.5 × 2.5–3 µm); pores small (4–6 per mm) ..... ***Xanthoporus peckianus***
    No thick-walled hyphae near the surface from stipe base; spores larger (4–5.6 × 3–4 µm); pores larger (3–5 per mm) ..... ***Xanthoporus syringae***
12. Spores elongated to cylindrical, 4.8–6.4 × 2–2.5 µm ..... ***Albatrellus pilosus***
    Spores not cylindrical 13
13. Spinose hyphae present on the mycelium and covering the stipe base; spores weakly amyloid 14
    No spinose hyphae; spores amyloid or not 15
14. Pileus variably colored when fresh (chamois, apricot, rarely brownish) but not blue ..... ***Albatrellopsis confluens***
    Pileus partly or entirely blue when fresh ..... ***Albatrellopsis flettii***

15. Pileus surface laccate . . . . . ***Albatrellus arizonicus***
    Pileus surface non-laccate 16

16. Pileus with black squamules and resinous dark zone between tubes and context . . . . . ***Albatrellus tianschanicus***
    Pileus without black squamules 17

17. Basidiome with numerous petaloid pilei from common base; pileus golden when fresh . . . . . ***Polypus dispansus***
    Basidiome with solitary pileus or confluent 18

18. Majority of spores longer than 5.5 µm . . . . . ***Laeticutis cristata***
    Majority of spores shorter than 5.5 µm 19

19. With hymeniderm; basidiome blue when fresh 20
    Without hymeniderm; basidiome not blue when fresh 21

20. Occurring in western North America; spores 3.6–4.7 × 2.9–3.7 µm . . . . . ***Neoalbatrellus subcaeruleoporus***
    Occurring in eastern North America; spores 4.6–5.6 × 3.8–4.3 µm . . . . . ***Neoalbatrellus caeruleoporus***

21. Spores inamyloid; associated with *Tsuga*, *Picea*, or *Abies* 22
    Spores distinctly amyloid; associated with especially *Pinus* or *Picea* 23

22. Spores 5–5.6 × 3.6–4.2 µm; under conifers, especially *Tsuga* and *Picea;* on west coast of North America. . . . . ***Albatrellus avellaneus***
    Spores 4.0–4.8 × 3.2–3.8 µm; under conifers, especially *Picea* and *Abies;* widely distributed. . . . . .***Albatrellus ovinus***

23. Under *Pinus;* basidiome yellow or orange where bruised or with age; taste bitter or mild . . . . . ***Albatrellus subrubescens***
    Under *Picea;* basidiome yellow where bruised; taste mild . . . . ***Albatrellus citrinus***